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16. ABSTRACT

Cadmium and zinc toxicity to the flagfish (Jordanella floridae) was determined on the basis of 96-hr median lethal concentrations (LC50) and significant decreases (P=0.05) in survival, growth, and reproduction over the complete life cycle of the fish. The 96-hr LC50 values for cadmium and zinc to juvenile flagfish were 2,500 and 1,500 µg/liter, respectively. In chronic tests, reproduction was the most sensitive indicator of cadmium toxicity and was inhibited at 8.1 µg/liter. Tissue-concentration analysis showed that fish exposed to concentrations of 1.7 µg/liter and above accumulated significantly greater amounts of cadmium than those in the controls. In zinc tests, survival of larvae (not exposed as embryos) and growth of females were the most sensitive measure of zinc toxicity and were reduced at respective concentrations of 85 and 51 µg/liter. Significant uptake of zinc occurred in fish exposed to concentrations of 47 µg/liter and above. The lowest cadmium and zinc concentrations causing adverse effects to the flagfish were similar to those affecting other fish species. Application factors for both metals were similar to those reported for cadmium exposed bluegills (Lepomis macrochirus) and zinc exposed fathead minnows (Pimephales promelas) in hard water.

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JOURNAL ARTICLE

Cadmium and Zinc Toxicity to flagfish, *Jordanella floridae*

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SPEHAR, R. L. 1976. Cadmium and zinc toxicity to flagfish, *Jordanella floridae*. J. Fish. Res. Board Can. 33: 1939–1945. 1976

Cadmium and zinc toxicity to the flagfish (*Jordanella floridae*) was determined on the basis of 96-h median lethal concentrations (LC50) and significant decreases ($P = 0.05$) in survival, growth, and reproduction over the complete life cycle of the fish. The 96-h LC50 values for cadmium and zinc to juvenile flagfish were 2500 and 1500 μg/liter, respectively. In chronic tests, reproduction was the most sensitive indicator of cadmium toxicity and was inhibited at 8.1 μg/liter. Tissue-concentration analysis showed that fish exposed to concentrations of 1.7 μg/liter and above accumulated significantly greater amounts of cadmium than those in the controls. In zinc tests, survival of larvae (not exposed as embryos) and growth of females were the most sensitive measure of zinc toxicity and were reduced at respective concentrations of 85 and 51 μg/liter. Significant uptake of zinc occurred in fish exposed to concentrations of 47 μg/liter and above. The lowest cadmium and zinc concentrations causing adverse effects to the flagfish were similar to those affecting other fish species. Application factors for both metals were similar to those reported for cadmium exposed bluegills (*Lepomis macrochirus*) and zinc exposed fathead minnows (*Pimephales promelas*) in hard water.

SPEHAR, R. L. 1976. Cadmium and zinc toxicity to flagfish, *Jordanella floridae*. J. Fish. Res. Board Can. 33: 1939–1945.

Nous avons mesuré la toxicité du cadmium et du zinc chez *Jordanella floridae* en nous basant sur les concentrations létales médianes après 96 h (CL50) et sur des diminutions significatives ($P = 0.05$) de survie, de croissance et de reproduction durant un cycle biologique complet du poisson. Les CL50 après 96 h de cadmium et de zinc pour des *Jordanella* juvéniles sont de 2500 et 1500 μm/litre respectivement. Dans des essais chroniques, la reproduction est l'indicateur le plus sensible de toxicité du cadmium et est inhibée à 8.1 μg/litre. L'analyse des concentrations tissulaires démontre que les poissons exposés à des concentrations de 1.7 μg/litre et plus accumulent des quantités de cadmium nettement supérieures à celles des témoins. Dans des essais au zinc, la survie des larves (non exposées comme embryons) et la croissance des femelles sont les indicateurs les plus sensibles de toxicité du zinc et sont toutes deux réduites à des concentrations respectives de 85 et 51 μg/litre. Il y a assimilation significative de zinc chez les poissons exposés à des concentrations de 47 μg/litre et plus. Les concentrations minimums de cadmium et de zinc ayant des effets nocifs sur *Jordanella* sont semblables à celles qui affectent les autres espèces de poissons. Les facteurs d'application des deux métaux sont semblables à ceux qui ont été signalés pour des crapets arlequins (*Lepomis macrochirus*) exposés au cadmium et pour des têtes-de-boule (*Pimephales promelas*) exposés au zinc dans de l'eau dure.

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CADMIUM and zinc occur simultaneously in the environment (Lingane 1966; Schroeder et al. 1967) and are toxic to fish (National Academy of Sciences 1973). Although most toxicity tests with fish have been short-term acute exposures, an effort is being made at the Environmental Research Laboratory-Duluth to determine more sensitive effects of toxicants through chronic exposures of fish over their complete life cycle. Commonly used species, such as fathead minnows (*P. promelas*), bluegills (*L. macrochirus*), brook trout (*Salvelinus fontinalis*), and rainbow trout (*Salmo gairdneri*), however, require long testing periods (5 mo–3 yr) to obtain complete life cycle data. Smith (1973) has proposed the use of the flagfish (*Jordanella floridae*) for rapid chronic bioassays because of its short generation time (6–8 wk) and other unique features. Little information has been shown on the effects of toxicants on this species in the literature (Foster et al. 1966, 1969).

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The purpose of this work was to determine the toxicity of cadmium and zinc to the flagfish by studying the effects of these metals on all developmental stages of the life cycle. In addition, this study was designed to evaluate the application-factor concept (Mount and Stephan 1967b) with a species not previously tested.

Materials and Methods

Water Characteristics

Untreated Lake Superior water was used in all tests at 25 ± 2 C. Chemical characteristics of the test water were analyzed weekly according to methods described by the APHA et al. (1971). Means and ranges (in parentheses) for these measurements were (milligrams/liter): dissolved oxygen, 8.3 (7.2–9.5); hardness, 44 as $CaCO_3$ (41–46); alkalinity, 42 as $CaCO_3$ (39–44); and acidity, 2.4 as $CaCO_3$ (1.5–3.4). The pH ranged from 7.1 to 7.8.

Exposure System

The exposure system consisted of a proportional diluter (Mount and Brungs 1967) which delivered five toxicant concentrations and a control to duplicate exposure chambers. Spawning chambers were glass aquaria, 30 × 60 × 30 cm, with a water volume of 43 liters. Larval chambers were 15 × 53 × 22 cm and contained 22 liters of water. Flow rate to each spawning chamber was 15 liter/h providing 95% replacement of test water every 9 h (Sprague 1969).

Fluorescent bulbs provided a light intensity of 50 lm at the water surface. An automatically controlled 16-h photoperiod was used.

Toxicant Solution

Stock solutions were prepared by dissolving reagent-grade $CdCl_2$ and $ZnSO_4 \cdot 7H_2O$ in 15 liters of distilled water and were introduced from a Mariotte bottle to the diluter by a chemical metering device (McAllister et al. 1972). Weekly composite samples of the test water were analyzed by a Perkin Elmer Model 403 atomic absorption spectrophotometer. Measurements for cadmium concentrations in the water are included in Table 1 and those for two zinc tests are shown in Tables 2 and 3. Analysis of variance showed that zinc measurements were the same for the two tests. Calibration curves were constructed by adding known amounts of cadmium and zinc to Lake Superior (control) water. Mean percent recovery and standard deviation for 16 spiked cadmium and zinc samples were 91 ± 0.8 and 100 ± 2.0, respectively.

Tissue Analysis

Whole fish from each concentration were analyzed for cadmium and zinc after 30 days of exposure and at the end of the test (approximately 100 days). For the cadmium test, 10 fish were analyzed per concentration except at 16 and 31 μg/liter where only 1 and 4 fish survived, respectively. Five fish were analyzed per concentration in each of the two zinc tests. A 95–100% recovery of the metals for 35 spiked tissue samples was obtained using a wet digestion, atomic absorption spectrophotometric analysis (Leonard 1971).

Biological Procedures

In chronic tests, enough embryos were incubated in each exposure concentration (cadmium test, zinc test 1) or in the controls (zinc test 2) to provide 30 1-day-old larvae to each chamber for the start of the tests. Exposed larvae and larvae not previously exposed as embryos were utilized for zinc exposures to determine the effect of embryo acclimation on larval sensitivity. To control fungus, all embryos were treated with metal-free malachite green (4 mg/liter) for 10 min during the first 3 days of incubation.

Larvae were fed brine shrimp nauplii 4 times a day for the first 30 days. After this time, all fish were randomly reduced to 15 per chamber and fed a diet of frozen brine shrimp twice a day and salmon starter granules once a day. The number of fish per chamber was further reduced to two males and five females after males began displaying territorial behavior, about the 60th day of the test.

Reproduction studies were started after 30 or more embryos appeared on any one spawning substrate and were continued for a period of 45 days. Spawning substrates were approximately 13 × 8 cm and made from 20-mesh stainless steel screen that was corrugated and wrapped in dark orlon yarn. After spawning, 30–50 embryos were removed from the substrates, placed in oscillating egg cups (Mount 1968), and incubated in the test water. All embryos not incubated for hatchability determinations were counted and discarded.

Twenty newly hatched (F_1) larvae from each toxicant concentration were randomly selected and transferred to duplicate larval chambers for 30-day growth and survival exposure. In the cadmium test, control larvae were also transferred for 30 days to concentrations causing adverse effects to parental fish.

Flow-through 96-h acute toxicity tests were conducted in the chronic-test diluter system with juvenile flagfish 4–5 wk old, according to methods described by the APHA et al. (1971). The 96-h LC50 values were derived by graphical interpolation and used in the determination of application factors (maximum acceptable toxicant concentration [MATC]/96-h LC50) (Mount and Stephan 1967b) for both metals.

Statistical Analysis

For statistical evaluation, survival, growth, reproduction, and cadmium tissue-accumulation data were subjected to an analysis of variance ($P = 0.05$) and Dunnett's two-tailed comparison of treatment means to control means ($P = 0.05$) (Steel and Torrie 1960). A two-way analysis of variance was applied

Table 1. Survival, growth, and reproduction of flagfish (*Jordanella floridae*) exposed to several concentrations of cadmium; *$P = 0.05$ (significantly different

TABLE 1. Survival, growth, and reproduction of flagfish (*Jordanella floridae*) exposed to several concentrations of cadmium; $*P = 0.05$ (significantly different from control according to Dunnett's procedure).

	Measured Cd concn (μg/liter)											
	31 ± 5.6[a]		16 ± 2.9		8.1 ± 2.0		4.1 ± 0.81		1.7 ± 0.52		0.11 ± 0.07 (control)	
	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
						30 days						
Survival (%)[c]	70	10	77	77	77	97	87	93	97	100	97	97
Mean total length (mm)	16 ± 1.5[d]	—	19 ± 1.6	18 ± 2.3	18 ± 0.7	20 ± 1.7	20 ± 1.9	18 ± 2.3	18 ± 3.5	17 ± 2.1	20 ± 1.0	19 ± 1.7
						100 days						
Males/females at termination	0/0	0/1	0/3	0/1	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5
Mean total length (mm)												
Male	—		—		49 ± 7.8 (4)		53 ± 1.7 (4)		52 ± 1.7 (4)		55 ± 3.3 (4)	
Female	31 (1)[d]		33 ± 2.1 (4)[d]		43 ± 3.2 (10)		43 ± 3.1 (10)		43 ± 2.2 (10)		45 ± 2.9 (10)	
Mean spawnings/female	0	0	1.7	1.0	5.2	3.0	10.4	8.6	11.0	11.4	8.8	8.4
Total embryos produced	0	0	163	35	1560	1270	3320	3280	4240	3440	3220	3430
Mean hatchability (%)	—		68		66		73		66		66	

[a]Mean ± SD.
[b]Duplicate chamber.
[c]Thirty fish per chamber.
[d]Number of fish analyzed.

TABLE 2. Survival, growth, and reproduction of flagfish (initially exposed as embryos) at several concentrations of zinc (test 1); $*P = 0.05$ (significantly different from control according to Dunnett's procedure).

	Measured Zn concn (μg/liter)											
	267 ± 28[a]		139 ± 18		75 ± 11		47 ± 11		28 ± 11		10 ± 13 (control)	
	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
						30 days						
Survival (%)[c]	10[e]	0	70	80	100	97	90	100	83	90	83	97
Mean total length (mm)	—	—	18 ± 2.2[e]	18 ± 0.9	18 ± 2.0	18 ± 1.4	19 ± 1.7	19 ± 1.8	18 ± 2.7	19 ± 2.0	18 ± 2.5	19 ± 2.0
						100 days						
Males/females at termination	0/0	0/0	0/4	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5	2/5
Mean total length (mm)												
Male	—	—	45 ± 5.7 (2)[e]		51 ± 4.5 (4)		55 ± 2.7 (4)		56 ± 3.4 (4)		54 ± 3.4 (4)	
Female	—	—	40 ± 6.0 (9)		43 ± 2.5 (10)		43 ± 1.9 (10)		43 ± 2.4 (10)		43 ± 2.2 (10)	
Mean spawnings/female	—	—	0.3	1.4	4.0	4.6	9.4	3.4	4.8	4.4	10.8	4.8
Total embryos produced	—	—	130	197	1310	1650	3230	995	1560	1240	3650	1196
Mean hatchability (%)		—	73		59		72		76		70	

[a]Mean ± SD.
[b]Duplicate chamber.
[c]Thirty fish per chamber.
[d]Number of fish analyzed.

TABLE 3. Survival, growth, and reproduction of flagfish (not exposed as embryos) at several concentrations of zinc (test 2); *$P = 0.05$ (significantly different from control according to Dunnett's procedure).

	Measured Zn concn (*µg/liter*)											
	267 ± 28[a]		139 ± 18		85 ± 11		51 ± 9		26 ± 8		< 1.0 (control)	
	A[b]	B[b]	A	B	A	B	A	B	A	B	A	B
30 days												
Survival (%)[c]	0*	0	0*	0	20*	23	82	97	93	93	93	87
Mean total length (*mm*)	—	—	—	—	—	—	22 ± 2.3*	21 ± 3.5	21 ± 1.7	22 ± 1.8	23 ± 2.4	22 ± 2.4
100 days												
Males/females at termination	0/0	0/0	0/0	0/0	2/3	3/4	2/5	2/5	2/5	2/5	2/5	2/5
Mean total length (*mm*)												
Male	—	—	—	—	52 ± 8.4 (5)[d]		53 ± 2.9 (4)		53 ± 1.7 (4)		54 ± 1.8(4)	
Female	—	—	—	—	36 ± 4.6 (7)*		38 ± 5.5 (10)*		40 ± 3.5 (10)		43 ± 2.5 (10)	
Mean spawnings/female	—	—	—	—	4.0	0.3	2.2	6.6	5.4	3.2	9.4	4.6
Total embryos produced	—	—	—	—	322	11	272	2440	1050	348	2320	1360
Mean hatchability (%)	—		—		88		83		83		69	

[a]Mean ± SD.
[b]Duplicate chamber.
[c]Thirty fish per chamber.
[d]Number of fish analyzed.

to zinc-accumulation data for comparisons between tests.

Results

Cadmium Toxicity

In 30 days, the highest concentration (31 μg/liter) retarded growth and appeared to reduce survival (Table 1). Survival also appeared reduced at the next lower concentration (16 μg/liter). However, due to the variability of response in duplicate chambers, statistical significant differences ($P = 0.05$) in survival could not be shown in any of the concentrations. Additional mortality occurred at 16 and 31 μg/liter after this period and by the 60th day, survival was less than 35% and growth of fish at 16 μg/liter appeared to be reduced. Cadmium-induced mortalities of fish were always preceded by periods of involuntary muscle spasms. At the end of the test (approximately 100 days) all males were dead in the two highest concentrations and growth of five surviving females was significantly reduced. Mean spawnings per female and embryo production were adversely affected at 8.1 μg/liter (Table 1). No significant differences in survival, growth, or reproduction were observed below 8.1 μg/liter.

Embryos from exposed parents incubated at 16 μg/liter and below hatched as well as those in the control (Table 1). Survival and growth of F_1 larvae were not adversely affected at these concentrations after 30 days of exposure; however, survival of larvae transferred at hatch from control water to 31 μg/liter was less than 20%. No adverse effects were seen when control larvae were transferred to 16 μg/liter and below for this period.

The 96-h LC50 value calculated for 4–5-wk-old juveniles was 2500 μg Cd/liter.

Fish exposed for 30 days to concentrations of 1.7 μg Cd/liter and above contained significantly higher amounts of cadmium than did those in the controls (Fig. 1A). Cadmium uptake increased with increasing exposure concentrations but leveled off at 16 μg/liter. Concentrations of cadmium (micrograms per gram) were lower in fish exposed for 100 days than those exposed for 30 days, but older fish contained a significantly higher total body content (micrograms per fish). In addition, cadmium concentrations in 30-day-old exposed parental fish and progeny (F_1) were similar.

Zinc Toxicity

Test 1 — Survival of larvae previously exposed to zinc as embryos was reduced at 267 μg/liter after 30 days (Table 2). After 100 days of exposure, all fish had died at this concentration and growth of males was less than those of the controls at the next lower concentration (139 μg/liter). Mean spawnings per female and embryo production appeared to be reduced at 139 μg/liter but significant differences could not be shown due to the variable response.

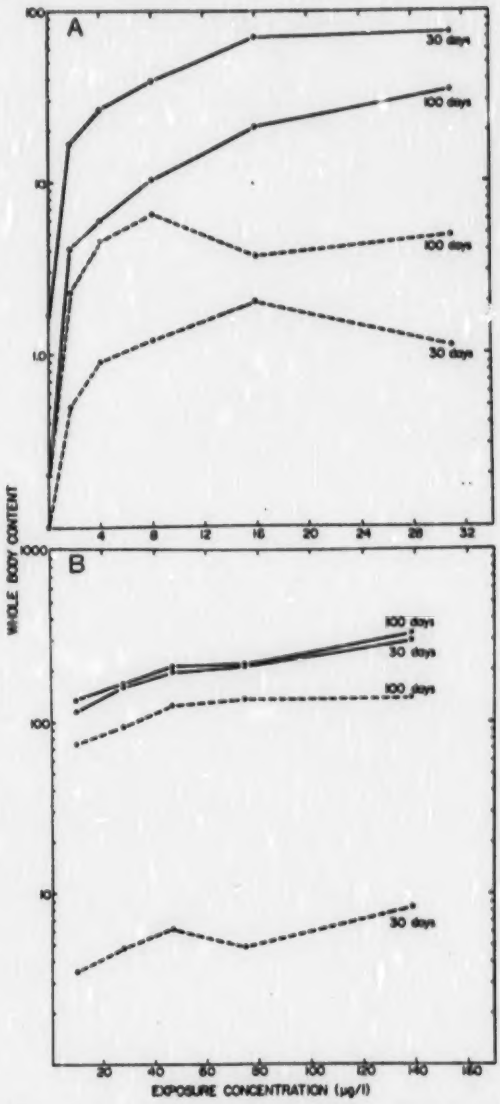

Fig. 1. Log cadmium (A) and zinc (B) content measured from whole body tissue of flagfish (*Jordanella floridae*) exposed to several cadmium concentrations for 30 and 100 days. Solid lines, micrograms per gram; dotted lines, micrograms per fish.

Test 2 — After 30 days, survival of larvae not previously exposed to zinc as embryos was adversely affected at 85 μg/liter (Table 3). No larvae survived at the two highest concentrations (139 and 267 μg/liter). By the end of the test, female growth was significantly lower than the controls at 85 and 51 μg/liter. Mean spawnings per female and embryo production appeared to be reduced at 85 μg/liter but were not statistically different from the controls.

Embryo hatchability (Tables 2 and 3) and survival and growth of F_1 larvae from exposed parents at 139 μg/liter and below were not significantly different from those of control larvae.

The 96-h LC50 value calculated for 4–5-wk-old juveniles was 1500 μg Zn/liter.

Fish exposed for 30 days to concentrations of 47 μg Zn/liter and above contained significantly higher amounts of zinc than those in the controls (Fig. 1B). Additionally, zinc uptake increased with increasing exposure concentrations. Concentrations of zinc (micrograms per gram) in fish exposed for 30 and 100 days were similar but older fish contained a significantly higher total body content (micrograms per fish). Zinc concentrations were also similar in 30-day-old exposed parental fish and progeny (F_1) and in fish previously exposed as embryos (test 1) and those not previously exposed (test 2) after 100 days.

Resulting Test Values and Application Factors

Results described above for chronic effects of cadmium and zinc on survival, growth, and reproduction establish the MATC (as described by Mount and Stephan 1967b) between 4.1 and 8.1 μg/liter cadmium and 26 and 51 μg/liter zinc. The application factor (MATC/96-h LC50) based on the 96-h LC50 values of 2500 μg Cd/liter and 1500 μg Zn/liter was between 0.0016 and 0.0032 for cadmium and 0.017 and 0.034 for zinc.

Discussion

Response of flagfish to sublethal concentrations of cadmium and zinc was different from those of other tested species. Pickering and Gast (1972) and Eaton (1974) found that embryos of fathead minnows and larvae of bluegills were the stages most sensitive to cadmium exposure. However, in the present study, spawning and embryo production were the most sensitive measures of effect. For zinc tests, survival of flagfish larvae and adult growth were adversely affected at lower concentrations than those affecting reproduction. This finding was different than the results of fathead minnow zinc exposures conducted by Brungs (1969). He found that reproduction was the most sensitive measure of zinc toxicity. Although the response of flagfish to cadmium and zinc was different from that of exposed fathead minnows and bluegills, the present results indicate that flagfish sensitivity was similar to that of cadmium-exposed brook trout (Benoit et al. 1976) and zinc-exposed rainbow trout (Sinley et al. 1974) tested in soft water.

In this test, larvae previously exposed to cadmium and zinc as embryos were more tolerant than those not previously exposed. This effect was much more pronounced in zinc tests as indicated by results shown in Tables 2 and 3. Sinley et al. (1974) reported that rainbow trout not exposed to zinc as embryos may be as much as 4 times more susceptible to zinc than fish previously exposed as embryos. Although the mechanism for this phenomenon is poorly understood, Wedemeyer (1968) showed that coho salmon (*Oncorhynchus kisutch*) eggs accumulate zinc highest in the chorion (70%), but that the developing embryos accumulate only a small amount (1%). Wedemeyer also pointed out that malachite green concentrations greater than 5 ppm increased zinc permeability of the vitelline membrane, thereby increasing the uptake of zinc in the yolk. Although the malachite green concentrations used to control fungal growth in the present tests were lower than those increasing zinc permeability, the danger exists that the toxicity of zinc was affected to some extent. No attempt was made to study the effects of this dye on zinc uptake in this test. The known action of malachite green on zinc and possibly other pollutants suggests, however, that it should not be used in toxicity studies.

Relationships between cadmium tissue uptake and water concentration in this test were similar to those observed by Mount and Stephan (1967a) for cadmium-exposed bluegills. The leveling-off of cadmium uptake at 16 μg/liter indicates that a possible equilibrium was reached between cadmium concentrations in the water and in the tissues. Comparisons between 30- and 100-day-exposed fish and accumulations expressed on a concentration (micrograms per gram) and total uptake (micrograms per fish) basis (Fig. 1A) show that cadmium accumulated with age, but at a rate that was slower than the growth of the fish. Similar results were indicated for the uptake of zinc (Figure 1B). Absence of significant additional metal uptake on a concentration basis was probably related to mechanisms affecting rates of uptake and elimination as was proposed by Coleman and Cearley (1974) for silver uptake in largemouth bass (*Micropterus salmoides*) and bluegills. Additionally, tissue concentration did not differ significantly between fish

exposed to zinc as embryos and those not previously exposed. This was attributed to the inability of zinc to penetrate the egg chorion to a large extent as was discussed by Wedemeyer (1968). Similar tissue concentrations in parents and progeny were probably the result of the same phenomenon. Experiments involving embryo and larval analyses would help to clarify mechanisms of metal uptake.

Abnormal behavior of exposed fish occurred at cadmium and zinc concentrations that caused death and inhibited reproduction. Cadmium especially had this effect on male fish during spawning. Eaton (1974) and Benoit et al. (1976) saw similar behavior in cadmium-exposed bluegills and brook trout. Abnormal behavior of bass and bluegills (uncontrolled swimming movements, convulsions, loss of equilibrium, and apparent coma) was also observed by Cearley (1971) when these fish were exposed to cadmium and silver. This type of behavior was attributed to the inhibition of acetylcholinesterase, causing death by paralysis of the muscles of respiration and/or depression of the respiratory center.

Flagfish application factors reported here for cadmium and zinc were similar to those reported by Eaton (1974) and Brungs (1969) for cadmium-exposed bluegills and zinc-exposed fathead minnows, respectively. Since bluegill and fathead minnow tests were conducted in hard water compared to the soft water of this test, water hardness, at least for these tests, would apparently not affect the estimation of chronic safe concentrations for these metals on the basis of calculated application factors.

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